eISSN 2255-0569

ORIGINAL

Examination of the Promotor Methylation of the *TRIM3* Gene in Obesity

Examen de la Metilación Promotora del Gen TRIM3 en la Obesidad

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Received: 11 - X - 2023
Accepted: 15 - XI - 2023

doi: 10.3306/AJHS.2024.39.02.87

Abstract

Background: Understanding the root causes of obesity, which is defined as having an increased amount of body fat, is crucial. Pathogenesis of obesity is influenced by environmental, genetic, and epigenetic factors.
Methods: The TRIM3 gene has been studied in this study to better understand how obesity develops. This study's goal was to determine the interaction between obesity and the methylation status of TRIM3.
Results: However, no statistically significant differences existed between the obese participants and the control group.
Conclusions: This study was the first to demonstrate how the TRIM3 gene and obesity interact, according to the literature.

Key words: TRIM3, methylation, obesity.

Resumen

Antecedentes: Es fundamental comprender las causas profundas de la obesidad, que se define como el aumento de la cantidad de grasa corporal. En la patogénesis de la obesidad influyen factores ambientales, genéticos y epigenéticos.
Metodología: En este estudio se ha estudiado el gen TRIM3 para comprender mejor cómo se desarrolla la obesidad. El objetivo de este estudio era determinar la interacción entre la obesidad y el estado de metilación de TRIM3.
Resultados: Sin embargo, no existían diferencias estadísticamente significativas entre los participantes obesos y el grupo de control.
Conclusiones: Este estudio fue el primero en demostrar cómo interactúan el gen TRIM3 y la obesidad, según la bibliografía.

Palabras clave: TRIM3, metilación, obesidad.

Introduction

The worldwide obesity issue is being highlighted by the World Health Organization in a number of ways. In order to manage and guard against obesity and overweight, a wide range of standards must be established. Aside from advice, nations must also receive assistance with implementation[1]. Nearly every system in the body is impacted by obesity. Since it has a detrimental effect on the reproductive and cardiovascular systems[2]. Genetics are a major contributor to obesity[2].

The first patients with congenital leptin insufficiency were discovered as a result of the discovery of leptin and its receptor genes, which were thought to be candidate genes for human obesity. Most of the obesity-related monogenic mutations have been found in populations of people with severe and early-onset obesity[3]. Common polymorphisms in candidate genes were examined for any potential associations with obesity incidence, BMI, or different aspects of body composition. The link between obesity outcomes and variations in candidate genes *ADRB3, BDNF, CNR1, MC4R, PCSK1*, and *PPARG* has been demonstrated to be consistent[4]. Several studies have found interaction between *PTH, FSHR*[5], *CLOCK*, *BMAL1*[6], *RANKL*[7] methylation and the obesity.

The broad, diverse, and ancient protein family known as TRIM proteins is involved in a variety of processes, including cellular differentiation, autophagy, apoptosis, DNA repair, and tumor suppression[8].

Cite as: Oztenekecioglu BH, Becer E, Kalkan R. Examination of the Promotor Methylation of The *TRIM3* Gene in Obesity. *Academic Journal of Health Sciences 2024*; 39 (2):87-91 doi: 10.3306/AJHS.2024.39.02.87

Apoptosis, cell cycle regulation, viral response, cell proliferation, oncogenesis, and antiviral defense are only a few of the physiological activities that TRIM proteins, which are present in humans in about eighty-fold abundance, are involved in. Their alteration inevitably results in a wide range of pathological illnesses, as well as problems with the heart, the brain, the immune system, the musculoskeletal system, development, and cancer[9]. Each disease, such as obesity, cancer, bacterial or viral infections, autoimmune diseases, developmental abnormalities, neuropsychiatric disorders, and congenital defects, are affected by TRIM proteins differently[10,11]. Obesity's etiology has been connected to TRIM3 expression. A CpG location in the TRIM3 gene shows lower methylation levels in obesity, according to a genome-wide methylation analysis of peripheral blood leukocytes[10]. In this study, we aim to determine whether there is a connection between obesity and the *TRIM3* gene's methylation state.

Material and Methods

Study Case

There were 59 obese and 62 non-obese participants in this study. The study's goal was to investigate the relationship between TRIM3 DNA methylation status and obesity. The Near East University's Scientific Research Ethics Committee approved the study, and informed permission was established among all participants (YDU/2021/96-1425).

Each patient was assessed prior to inclusion for the presence of malignancy, diabetes mellitus, hypertension, dyslipidemias, liver cirrhosis, thyroid, cardiovascular, or any active inflammatory condition. In this study, professional athletes were not included. All subjects completed written informed consent forms and provided information about their medical histories. The Near East University's Research Ethics Committee gave its approval to the study protocol, which was carried out in conformity with the Helsinki Declaration.

Determination of the Biochemical Parameters

After a 12-hour fasting, peripheral blood samples from the subjects were collected in the morning. A fully automated clinical biochemistry analyzer was used to measure the levels of serum fasting glucose, triglycerides (TG), total cholesterol, high-density, low-density lipoprotein cholesterol (LDL-C), and lipoprotein cholesterol (HDLC) (Abbott Architect C8000). Additionally, serum levels of leptin, resistin, and adiponectin were measured using ELISA kits in accordance with manufacturer's instructions (DRG Intl., Inc., USA for leptin and Biovendor Laboratory, Inc., Brno, Czech Republic for resistin and adiponectin).

Methylation Sensitive High-Resolution Melting Analysis

Patients with obesity and patients with normal controls had their peripheral blood samples taken. The AllPrep DNA/RNA/Protein isolation kit was used to isolate DNA (Qiagen in Manchester, United Kingdom). A NanoDrop ND-1000 Spectrophotometer was used to measure the DNA content (Thermo Fisher Scientific, Waltham, MA USA). The sodium bisulfite modification was done using the Epitect Bisulfite Modification Kit from Qiagen in Manchester, UK. Based on the MS-HRM PCR Guide, a collection of primer sequences was created. (Qiagen, Manchester, UK). EpiTect was used to determine the methylation status of the TRIM3 promotor. MS-HRM PCR Handbook guidelines (Rotor-Gene Q, Qiagen)7.

Statistical Analysis

For data analysis, GraphPad Prism 7 software was employed. For continuous variables, data in tables are shown as mean ± standard deviation (SD), and for discrete variables, absolute values (percentages). A statistically significant difference was defined as $p<0.05$.

Results

Patients with obesity had a mean average age of 43,58±11,21 years and a BMI of 36,31±7,86 kg/m^2. The mean age of control group was 42,16±13,68 years and their mean BMI was 23,66±2,45 kg/m^2. **Table I** displays the anthropometric and metabolic features of the patients.

Table I: The Anthropometric and Metabolic Characteristics of Studied Patient Population.

Parameter	Non-obese subjects			Obese subjects		
	Methylated (31)	Unmethylated (31)	p	Methylated (24)	Unmethylated (35)	p
Age	42,16±13,68	41,35±13,09	0,81	43,58±11,21	44,34±9	0,77
BMI (kg/m²)	23,66±2,45	23,81±2,68	0,82	36,31±7,86	34,4±4,57	0,24
Waist circumference (cm)	83,52±8,17	84,94±8,69	0,51	117,9±15,65	112,5±13,14	0,15
Hip circumference (cm)	100,5±7,27	99,52±6,80	0,57	120,3±10,14	119,6±10,05	0,79
Fasting glucose (mg/dL)	88,16±7,74	89,19±5,64	0,55	101,5±18,64	104,3±22,85	0,61
Total cholesterol (mg/dL)	202±25	202,7±22,14	0,91	222,7±29,71	224,6±40,5	0,84
LDL-cholesterol (mg/dL)	127,6±25,83	130,7±25,73	0,63	137,4±25,77	138,5±34,31	0,89
HDL-cholesterol (mg/dL)	56,74±7,35	58,39±10,76	0,48	43,42±7,50	47,4±9,98	0,1
Triglycerides (mg/dL)	100,5±34,91	90,35±40,59	0,29	177,8±73	162,1±67,19	0,39
HOMA-IR	1,94±0,55	1,91±0,62	0,81	4,02±2,26	4,23±2,44	0,73
Leptin (ng/ml)	8,94±3,71	9,6±6,47	0,62	23,42±14,07	21,04±10,81	0,46
Adiponectin (µg/mL)	18,86±8,93	22,03±9,14	0,17	10,54±4,96	10,39±5,15	0,91
Resistin (ng/mL)	6,303±2,32	5,99±3,02	0,64	8,85±2,37	8,74±2,68	0,87

Determination of *TRIM-3* gene methylation pattern

In 24 out of 59 obese patients (40,68%) and 31 out of 62 non-obese people (50%) the TRIM-3 gene was methylated. **Figure 1** shows that there was no methylation status–obesity relationship that was statistically significant ($p > 0.05$). Methylation status of TRIM-3 in obese and control subjects has been shown in **table II**.

Table II: Distribution of *TRIM-3* methylation.

Subjects	TRIM gene		
	Methylation	Unmethylation	p Value
Obese Non-obese	40,68% 50%	59,32% %50	0,36

The universally methylation control of the *TRIM3* gene was displayed as blue, whereas the universally unmethylated control was displayed as purple. Temperature (^{0}C) is plotted along the X-axis, and fluorescence (dF/DT) is displayed along the Y-axis. The averaged melting curves of the dilution standards demonstrate the optimization process in the TRIM3 promoter region (**Figure 1**).

Relationship between TRIM3 methylation level, anthropometric, and metabolic characteristics

Investigations were conducted into the associations between TRIM3 methylation status and measurements of the waist, hips, BMI, age, insulin concentration, HOMA-IR, leptin, adiponectin, and resistin as well as levels of circulating glucose, triglycerides (TG), total cholesterol, HDL-C, and LDL-C. However, there was no evidence of a significant relationship between anthropometric or metabolic traits and TRIM3 methylation status (**Table III**).

Discussion

The development of obesity is significantly influenced by the interaction between genetics and epigenetics. Several studies have shown a relationship between obesity and epigenetic changes. An epigenetic mechanism called DNA methylation contributes to the development of obesity and its metabolic side effects[7,12].

Epigenetic changes in the following genes have been linked to obesity: CLOCK, BMAL1, PER2, UBASH3A, TRIM3, LEP, ADIPOQ, PGC1, IGF-2, IRS-1, LY86, MEST, PEG3, NNAT, PLAGL1, MEG3, NPY, IL6, TNF, TFAM, GLUT4, RANKL, and c-FOS[7,13,14,15,16]. Several studies have found interaction between PTH, FSHR[5], CLOCK, BMAL1[6], RANKL[7] methylation and the obesity.

A genome-wide methylation investigation revealed that TRIM3 methylation levels were decreased in obese individuals[17]. The DNA methylation pattern in peripheral blood leukocytes of obese people was identified using a genome-wide strategy.

Figure 1: Increasing The Annealing Temperature Improves Assay Sensitivity When PCR Primers Include CpG Dinucleotides.

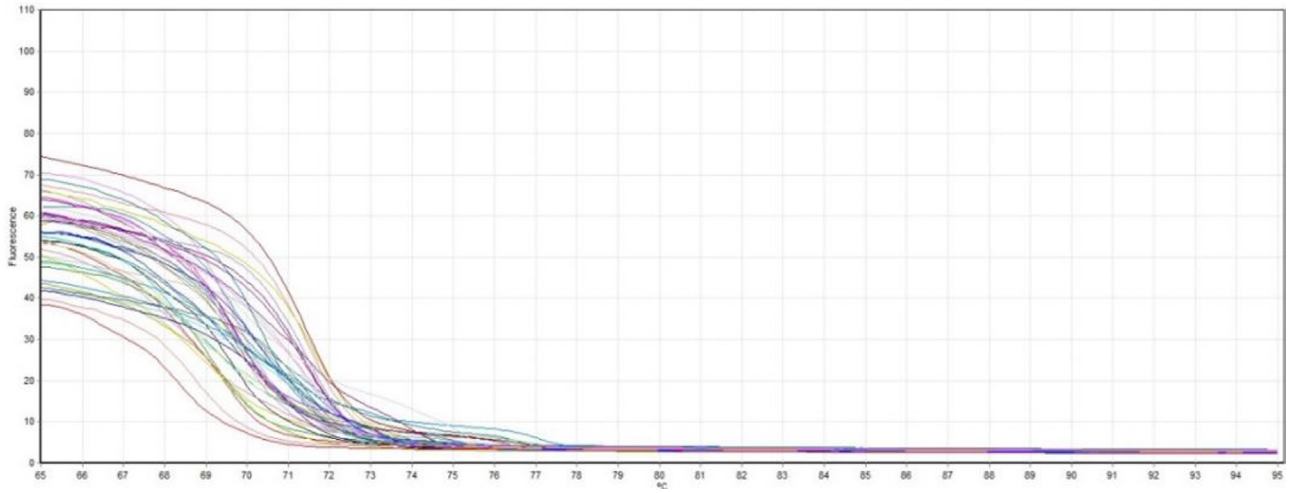

Table III: *TRIM3* gene and obesity status.

TRIM3		OBESE STATUS			
		nonobese	obese	total	p-value
unmethylated	observed %within column	31 50.0%	35 59,32%	66 66.0%	p>0,36
methylated	observed %within column	31 50.0%	24 40,68%	55 55.0%	
Total	observed %within column	62	59	121	

A genome-wide approach was used to define the DNA methylation pattern in peripheral blood leukocytes of obese subjects. Significant changes in CpG methylation were detected between obese participants and controls in the genome-wide methylation investigation. Both the monitoring and replicating cohorts showed reduced methylation of the promoter CpG island in obese individuals[17,10]. According to Wang et al. (2010), obese subjects had altered methylation patterns at one CpG site in the UBASH3A gene and one in the TRIM3 gene, respectively, both during the genome-wide analysis and the verification process (P = 0.008 and P = 0.001 for the UBASH3A and TRIM3 genes, respectively)[17].

Despite the fact that there is evidence connecting obesity with TRIM3 methylation, little is understood about the precise mechanisms at play. We hypothesized that DNA methylation might contribute to obesity based on growing evidence that epigenetic regulation regulates gene expression. The aim of this study was to investigate whether or not methylation of the TRIM3 gene is associated with obesity.

Patients with obesity had a mean age of 43,58±11,21 years and a BMI of 36,31±7,86 kg/m^2. The average age and BMI of the control group were 42,16±13,68 years and 23,66±2,45 kg/m^2, respectively. In 24 out of 59 obese patients (40,68%) and 31 out of 62 control subjects (50%) the *TRIM-3* gene was methylated. There was no statistically significant association between obesity and methylation status ($p > 0.05$). In 35 out of 59 obese patients (59,32%) and 31 out of 62 non-obese people (50%) had the *TRIM-3* gene unmethylated. Unmethylation status and obesity were not statistically different from one another ($p > 0.05$).

In this study, the methylation and unmethylation status of the *TRIM3* gene were compared to the mean age of the individuals. The TRIM3 gene's mean age was 44,34±9 (mean ± Std. Deviation), while the methylated TRIM3 gene's mean age was 43,58±11,21 (mean ± Std. Deviation). The statistical analysis revealed no significant associations between the age of the obese participants and their TRIM3 gene methylation status ($p = 0.77$).

This research presents evidence that obesity is linked to DNA methylation modifications by identifying differences in the TRIM3 methylation status between obese participants and control controls. Obese subjects have excessively high leptin levels. Leptin resistance is the term used to describe this phenomenon[18]. In obese individuals, visceral body fat may have an impact on medical issues through an abnormal adipokine production. The concentration of total adiponectin and adiponectin with a high molecular weight decreases in obesity and increases after weight loss, indicating that adiponectin plays a critical role in energy homeostasis. Adiponectin production and release may be impacted by the growth of adipose tissue[19]. Extreme insulin sensitivity has been linked to higher levels of resistin in humans than conventional insulin action. Resistin may therefore be crucial in the development of insulin resistance[20].

In our study, the methylated *TRIM3* participants had a mean leptin level of 21,04±10,81 (mean ± Std. Deviation) while the unmethylated *TRIM3* subjects had a mean leptin level of 23,42±14,07 (mean ± Std. Deviation) ($p = 0,46$). Unmethylated TRIM3 patients had a mean level of adiponectin of 10,39±5,15 (mean ± Std. Deviation,), whereas methylated TRIM3 subjects had a mean level of 10,54±4,96 (mean Std. Deviation) ($p = 0,91$). The mean levels of resistin were 8,74±2,68 (mean Std. Deviation) in the unmethylated TRIM3 patients and 8,74±2,68 (mean ± Std. Deviation) in the methylated TRIM3 subjects ($p = 0,87$). As a result, there was no statistically significant correlation between anthropometric or metabolic traits and TRIM3 methylation status (**Table I**).

Conclusion

An important part of obesity is played by genetic and epigenetic factors. The importance of the interaction between genetic and environmental variables on the etiology of obesity is highlighted by studies and changes of epigenetic regulators. Numerous obesity susceptibility genes have been found by researchers, along with their function in the progression of disease.

Accordingly, epigenetic changes and how they interact with the environment have the potential to become biomarkers for obesity. According to these findings, a number of studies have looked into possible biomarkers of obesity in an effort to raise the quality of life for those who are affected, lessen their symptoms, assist them in adjusting to daily life, and strengthen and improve their attitudes towards interpersonal interactions. This study was unable to find a connection between obesity and the TRIM3's methylation pattern. There haven't been many studies on epigenetics in this field. Our research thus clarifies epigenetics and provides crucial data for next investigations, such as those looking for biomarkers for obesity.

Authors Contribution

Conceptualization: RK; Methodology: RK and BO; Software: RK and BO; Validation: RK; Formal analysis: RK, and BO ; Investigation: RK and BO; Resources: RK, BO, and EB; Data curation: RK, and BO; Writing—original draft preparation: RK, and BO;
Writing—review and editing: RK, BO, and EB; Visualization: RK, BO, and EB; Supervision: R.K.;

Project administration

RK; Funding acquisition: RK.

Funding
The authors received no specific funding for this work. We would like to thank all the participants involved in this study.

Data Availability
The genetics data that support the findings of this study are available on request from the corresponding author (RK).

Declarations
Conflict of Interest
The authors affirm that they do not have any competing interests.

Ethical Approval
All subjects signed written consent forms after being fully informed. The study protocol was approved by the Research Ethics Committee of the Near East University and performed in accordance with the Declaration of Helsinki (YDU-2021/96–1425).

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